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Farnesol, psycho-sedative and spasmolytic substance

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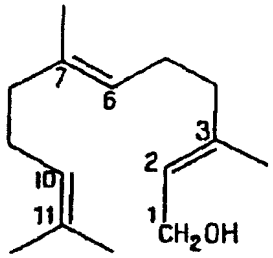
Certain terpene alcohols (citronellol, farnesol, geraniol, linalol, nerol, nerolidol, rhodinol, dehydrolinalol) reduce the motility of mice and oppose the acetylcholine spasm of the Rat duodenum [2], modify the behavior of the Goldfish and temporarily suppress the aggressiveness of the Fighting Fish [3]. It therefore seemed worthwhile to us to study through other tests the most active among them, farnesol, in order to place it among the psychotropic substances.

The farnesol molecule (trimethyl-3,7,11 dodecatriene-2,6,10 ol-1) can exist in four stereoisomeric forms (trans-trans, cis-cis, trans-cis and cis-trans) the structure of which is linked to the configuration of the double bonds 2-3 and 6-7.



The farnesols of natural or synthetic origin generally are mixtures containing at least two of the expected stereoisomers, and the isolation of the latter is really feasible only by preparative gas chromatography on small quantities. We therefore worked on farnesol prepared by synthesis according to L. RUZICKA [15]. In the latter, the farnesol fraction contained approximately 10 p. 100 of cis-cis, 44 p. 100 of cis-trans and 46 p. 100 of trans-trans. There also was a small fraction of impurities (on the order of 5 p. 100)

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consisting mainly of nerolidol. The identification of the different isomers is based on the retention time in gas chromatography (Aerograph Apparatus A-90-P, Carbowax 20 M column on Chromosorb E, column temperature 210°) by comparison with the results of several authors [**1, 16, 17**]. The trans-cis isomer was not individualized, but might be carried along with the cis-trans isomer.

Farnesol, insoluble in water, was used either in aqueous suspension stabilized with addition of 1 p. 100 of tween 80, or in solution, assaying 20 to 50 p. 100 of active principle, in an excipient of oily consistency made up of triglycerides of saturated fatty acids with average length chain - 8 to 12 carbon atoms - "Miglyol 812 Witten," supplied by the company H. VILCOCQ, 9, Avenue de l'Opera, Paris (1st ward). In all that follows, these two preparations are designated respectively by the terms "suspension" and "solution."

ACUTE TOXICITY

It has been studied on the Mouse and evaluated on the Rat and the Rabbit. The results are as follows:

Mouse,	orally:	solution	LD_{50}	> 12 g/kg
		suspension		7.4 ± 0.6 g/kg
Mouse,	i.p.:	solution		2.95 ± 0.3 g/kg
		suspension		443 ± 42 mg/kg
Rat,	orally:	solution		> 10 g/kg
		suspension		6 to 10 g/kg
Rabbit,	orally:	solution		no death at 5 g/kg

Observation of the animals showed that, for the high doses, symptoms of prostration and sedation are manifested with the solution as well as with the suspension, but the latter seems more toxic. This last point might result in part from a slower absorption in the oily excipient, and in part from a certain toxicity of the tween 80 [**12**].

GENERAL SEDATIVE ACTION

Observation of the animals at the time of the acute toxicity studies, cited hereinabove, as well as what was noted on the Goldfish (*Carassius auratus*) [**3**] show that this substance is endowed with the general sedative properties. Which is confirmed by the hypothermia test in the Rat.

Hypothermia in the Rat.

Male rats from 200 to 275 g are placed individually in a parallelepiped box 19 x 11 x 6 cm, provided with holes in order to allow a suitable passage of ambient air. One opening is provided in order to keep the animal's head outside the box, and another for

the passage of a thermo-electric rectal probe. Under these conditions, the temperature of the animals is determined at time zero. Any animal having a temperature below + 36.5° or above + 38° is eliminated. Then the rats, each one in its box, are placed in the refrigerator at + 4°. After a stay of one hour, the rectal temperature is determined again, then the product is injected and the temperature of the animals kept in the refrigerator during the following 4 hours is determined. A comparison is made with the controls placed under the same conditions.

Four batches of 5 rats each are used for this test. One batch serves as a control (in the refrigerator, but no farnesol), the three other batches receive respectively 50, 100, 150 mg/kg of farnesol in suspension intraperitoneally. The results are indicated on Figure 1. They show that, under these conditions, farnesol is hypotherming and that its action is proportional to the dose administered.

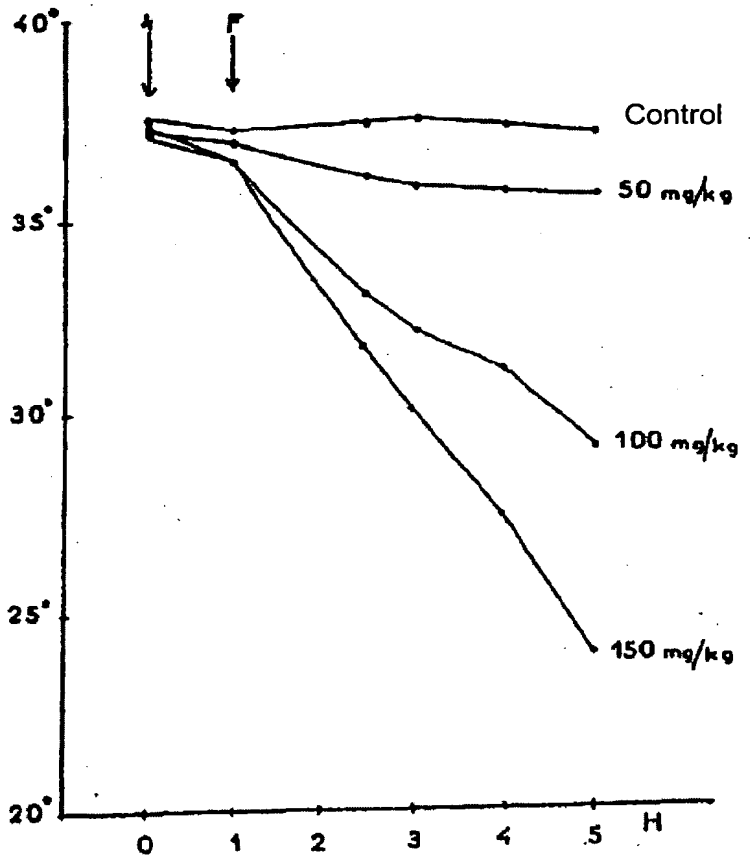

FIG. 1. - Action of farnesol on the rectal temperature of the Rat exposed to the cold.
On the abscissa, time in hours; on the ordinate, rectal temperature. At 4 (time zero), the animals are placed in the refrigerator at + 4°; at F, injection of farnesol except for the controls.

PSYCHO-SEDATIVE ACTION

Nine tests were carried out in order to clarify the features of the psychotropic action of farnesol.

1) Fighting-Fish test.

One of us studied the action of farnesol on the aggressiveness of the male Fighting Fish (*Betta splendens*), and observed a very marked inhibition thereof [3].

2) Actography.

In a prior work [2] it was indicated that the intraperitoneal injection of farnesol at the dose of 100 mg/kg in solution reduces for 120 minutes the motility of mice placed in an actograph. We confirm this result here and we extend it to the suspension administered orally or intraperitoneally and to the solution administered orally:

— suspension, orally: marked action starting from 150 mg/kg;
— solution, orally (*Fig. 2*): marked action for more than 2 hours at the dose of 100 mg/kg;
— suspension, i.p.: action discernible from 10 mg/kg, at 50 mg/kg action for 2 hours, at 100 mg/kg very significant action for more than 3 hours.

It was verified that the excipients alone have no action.

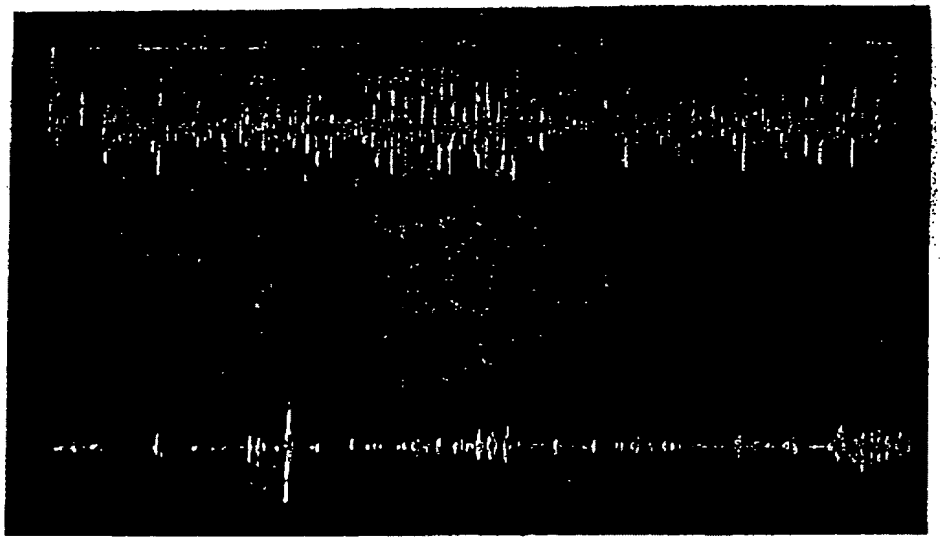

FIG. 2. - Actograph of control mice (at top) and of mice having ingested 100 mg/kg of farnesol 1 hour prior to the test (at bottom).

3) Curiosity test known as "board with holes" test.

This test was carried out according to the technique of J.-R. BOISSIER, P. SIMON and J.-M. LWOFF [8] under various experimental conditions: administration of farnesol in solution and in suspension, orally and intraperitoneally, the start of the test being set at a more or less greater time after administration of the product. The controls receive only the excipient. For each experiment, the percentage of holes explored by the treated animals in relation to the number of holes explored by the controls was calculated.

The results are grouped in Table I. They show that farnesol, irrespective of the form and manner of administration, reduces the curiosity and exploration response of mice.

TABLE I

Farnesol. Curiosity or "board with holes" test Percentage of holes explored in 5 minutes in relation to the controls	
Controls	**100**
Farnesol in solution 100 mg/kg orally	**57.6**
Farnesol in solution 100 mg/kg i.p.	**66.9**
Farnesol in suspension 100 mg/kg orally	**63.8**
Farnesol in suspension 100 mg/kg i.p.	**59.9**

4) Catalepsy.

The search for a possible cataleptigenic action was investigated according to the technique of J.-R. BOISSIER and P. SIMON [6] in which the behavior of the Rat is observed when the forelegs are crossed with the homolateral hind legs. Following administration of farnesol at doses of 50 and 100 mg/kg orally and intraperitoneally, this test is repeated every half-hour for 8 hours. A control test was carried out with intraperitoneal injection of tween alone.

In all cases, no cataleptigenic effect could be demonstrated, which clearly differentiates farnesol from the neuroleptics.

5) "Pull-up" or "chinning bar" test.

This test, known as JULOU and COURVOISIER [4], consists in suspending mice by their forelegs on a horizontal metal rod. Those which, in 5 seconds, perform a pull-up that brings at least one of the hind legs up to touch the wire, are selected. The mice, in batches of ten, receive intraperitoneally an injection of farnesol suspension. The dose of farnesol varies from 100 to 400 mg/kg. Controls receive tween alone.

The controls do a pull-up in less than 5 seconds.

All the treated mice accept hanging onto the bar, which differentiates farnesol from the hypnotics.

All the mice treated with 100 mg/kg of farnesol do a pull-up in less than 5 seconds (no action), one out of ten takes more than 5 seconds to do a pull-up with 200 mg/kg of farnesol, two out of ten with 300 mg/kg, four out of ten with 400 mg/kg. This test therefore is positive only in the vicinity of the lethal doses.

6) "Chimney" test.

This test was advocated by J.-R. BOISSIER, J. TARDY and J.-C. DIVERRES [9]. It consists in observing the backwards climbing of a mouse in a narrow tube positioned vertically. The authors indicate that the mice treated with tranquilizers are not able to climb in the tube, while the control mice climb in a given time. The ED_{50} at time *t* is evaluated, that is, the dose that inhibits climbing back up for 50 p. 100 of the animals *t* minutes after administration of the substance to be studied. Here, farnesol in suspension was administered intraperitoneally.

The results are as follows (*Table II*).

It should be noted that most of the treated mice that nonetheless were able to climb back up took longer to do so than the control mice.

Under these conditions, the ED_{50} can be evaluated about one hour after the injection at 150 mg/kg.

TABLE II

"Chimney" test			
Dose administered	Number of animals	N 30	N 60
Controls.....	10	0	0
100 mg/kg (1st test).....	10	2	1
100 mg/kg (2nd test)	10	2	2
150 mg/kg.....	10	4	5
200 mg/kg.....	10	7	8
300 mg/kg.....	10	10	10

N 30: number of animals that do not climb back up when the test is performed 30 minutes after the injection of farnesol.

N 60: number of animals that do not climb back up when the test is performed 60 minutes after the injection of farnesol.

7) Action on the stimulation produced by caffeine.

In the Mouse, the administration of caffeine leads to a significant stimulation. According to J.-R. BOISSIER and P. SIMON [7], the stimulant doses are situated between 8 and 64 mg/kg orally, with a maximum effect at 16 mg/kg. We investigated

whether mice treated with caffeine could be calmed down with farnesol. This study was carried out on mice of Swiss strain.

During a preliminary test, in which stimulation was demonstrated by actography, it was observed, on groups of 6 animals, that the hypermotility generated by the ingestion of 50 mg/kg of caffeine was reduced considerably by the intraperitoneal injection, 30 minutes later, of 100 mg/kg of farnesol in suspension.

A more complete test, using the method of the board with holes, then was carried out according to the following protocol.

Caffeine, at set doses of 25 and 50 mg/kg, is given to two groups of mice orally (gummy suspension at 3 p. 100) two days in succession. Thirty minutes after the second administration, the mice are placed on a board with holes. Two other groups of mice are prepared in the same manner, but thirty minutes after the second administration, they receive an intraperitoneal injection of 100 mg/kg of farnesol in solution, and then are placed on the board with holes thirty minutes after this injection. One group of control mice, receiving neither caffeine nor farnesol, is placed for comparison on the board with holes. The results are set forth on Table III.

It appears that the stimulation produced by 25 mg/kg of caffeine is greater than that produced by 50 mg/kg, which is in conformity with the observations of J.-R. BOISSIER and P. SIMON [7]. As for the mice treated with caffeine, then farnesol, they are clearly calmer than those treated with caffeine alone, and even calmer than the control mice.

Under these experimental conditions, the psychic hyperstimulation produced by the caffeine is amply offset by the farnesol.

TABLE III

Farnesol-caffeine antagonism
Curiosity test
Percentage of holes explored in 5 minutes in relation to the controls

Controls (neither caffeine nor farnesol)	100
Caffeine 25 mg/kg	141
Caffeine 25 mg/kg + farnesol 100 mg/kg i.p.....	65
Caffeine 50 mg/kg	121
Caffeine 50 mg/kg + farnesol 100 mg/kg i.p.....	67

8) Action on pentetrazol convulsions

It was investigated whether farnesol modifies the convulsive action of pentetrazol, the latter being administered in slow venous injection in the Mouse according to the technique of H.-C. HINT and A.-W. RICHTER [11]. The farnesol is administered in intraperitoneal injection or orally, in the form of suspension, at doses of 100 or 200 mg/kg 30 or 60 minutes prior to injection of pentetrazol. In all cases, the development

and intensity of the convulsive attack that precedes death are not modified in the treated animals, only the minimum lethal dose is more or less increased, and as a result death occurs slightly later in the treated animals, but the differences are small (for example, the minimum lethal dose of pentetrazol increases from 117.7 to 150.3 mg/kg when the animals receive 100 mg/kg of farnesol intraperitoneally 30 minutes prior to the start of the pentetrazol injection). Orally, the effects of farnesol on this test are not significant. This seems to indicate, therefore, that farnesol cannot be regarded as an antagonist of pentetrazol convulsions.

9) Action on sleep induced by barbiturates.

We did not demonstrate any hypnotic action of farnesol, and we indicated that it behaves differently from the hypnotics with respect to the "chinning bar" test. But it intensifies the hypnotic action of the barbiturates, as can be demonstrated by two standard methods: on the one hand, prolongation of the duration of sleep and, on the other hand, "falling asleep again."

Method of prolongation of duration of sleep.

A hypnotic dose of mebubarbital is injected into two batches of mice: one control batch and one batch treated beforehand with farnesol; the duration of sleep of the two batches is compared. The farnesol is administered in suspension intraperitoneally 10 or

TABLE IV

Prolongation, by farnesol, of the duration of sleep induced by 35 mg/kg of mebubarbital	
Number of animals	*Average duration of sleep in minutes*
Farnesol injected 10 minutes prior to mebubarbital:	
Excipient alone..... 15	30
Farnesol 50 mg/kg..... 16	30.5
Farnesol 100 mg/kg..... 16	56.4
Farnesol injected 30 minutes prior to mebubarbital:	
Excipient alone:..... 8	31.3
Farnesol 50 mg/kg..... 16	31.4
Farnesol 100 mg/kg..... 8	90
Farnesol ingested 60 minutes prior to mebubarbital:	
Excipient alone..... 27	20
Farnesol 25 mg/kg..... 22	35
Farnesol 50 mg/kg..... 28	34.4
Farnesol 100 mg/kg..... 23	29.1

30 minutes prior to injection of the barbiturate or orally 60 minutes prior to the hypnotic. The controls receive the excipient alone. The mebubarbital is administered to all the animals intraperitoneally at the dose of 35 mg/kg in a solution assaying 5 mg/ml of product. The duration of sleep is assessed by observation of the straightening-up reflex **[4, 14]**. The results are grouped in Table IV.

It thus is noted that farnesol intraperitoneally at doses of 100 and 200 mg/kg clearly prolongs the duration of barbituric sleep, but that it has no action at the dose of 50 mg/kg. Orally, the duration of sleep is increased but does not seem to be in accordance with the dose administered.

Method of "falling asleep again."

This method, less sensitive than the preceding one, is truly specific for psycho-depressants **[14]**. The test is conducted as follows: male rats receive an intraperitoneal injection of mebubarbital at the dose of 35 mg/kg in solution at 0.5 p. 100 in sodium chloride at 9 p. 1,000. The time that elapses before falling asleep, the duration of sleep, are noted. Then one minute after the awakening, farnesol in suspension is injected intraperitoneally. The time that elapses before falling asleep again, then the duration of the second sleep, are noted.

$$\text{The ratio} \quad \frac{\text{duration of second sleep}}{\text{duration of first sleep}} = R \text{ is determined.}$$

The average results are collected in Table V.

This last test, very specific, shows that farnesol induces "falling asleep again" in rats at the end of barbituric sleep, as do many psycho-sedatives.

TABLE V

Falling asleep again after injection of farnesol Initial injection of 35 mg/kg of mebubarbital			
Average duration of first sleep	then injection of	Average duration of second sleep	R
91 minutes	Excipient alone	No second sleep	0
57 minutes	Farnesol 50 mg/kg	32 minutes	0.56
54 minutes	Farnesol 100 mg/kg	26 minutes	0.48
81 minutes	Farnesol 200 mg/kg	86 minutes	1.06

SPASMOLYTIC ACTION

The spasmolytic action of farnesol was investigated on the intestine and on the gallbladder.

1) Action on the isolated intestine.

The spasm-inducing agents used were: acetylcholine, barium chloride, histamine and serotonin. Farnesol was added to the bath in the form of a suspension. The preventive action, or the curative action, was investigated. In all cases the control tests were carried out with the excipient alone, because it is know that tween 80 in itself has spasmolytic properties. [**12**].

a) *Spasm induced by acetylcholine.*

In the isolated duodenum of the Rat, taking into account the action of tween 80 itself, it appeared that the spasm induced by 0.05 µg/ml of acetylcholine is reduced by 50 p. 100 by prior administration of 7 to 10 µg/ml of farnesol, or by subsequent administration of 1.5 to 2.5 µg/ml of this same substance.

b) *Spasm induced by barium chloride.*

Under the same conditions, the spasm induced by 50 µg/ml of barium chloride is reduced by 50 p. 100 by prior administration of 7 µg/ml or administration of 2.75 to 6 µg/ml of farnesol.

c) *Spasm induced by histamine.*

On the guinea-pig ileum, in an atropine medium, the spasm induced by histamine at the concentration of 2 to 4.10 [illegible] is reduced by 50 p. 100 by 2.5 µg/ml of farnesol.

d) *Spasm induced by serotonin.*

On the isolated Rat colon, according to the technique of D. QUIVY [**13**], the spasm induced by 0.03 µg/ml of serotonin is reduced by 50 p. 100 by 1.3 µg/ml of farnesol.

2) Action on the isolated guinea-pig gallbladder.

The spasm induced by 0.05 µg/ml of acetylcholine is reduced by 50 p. 100 by 5 to 20 µg/ml of farnesol.

3) Action on the gallbladder and the Oddi sphincter of the Guinea-pig "in situ."

According to the technique of perfusion under constant pressure of J. R. BOISSIER and J. J. CHIVOT [**5**], a spasm of the Oddi sphincter is induced by

intravenous injection of 5 to 10 μg/ml of carbamoylcholine. This spasm is prevented or reduced by intravenous injection of a suspension of farnesol at the dose of 5 to 25 mg/kg.

Farnesol therefore appears to be a spasmolytic substance for the smooth fibers, in all probability musculotropic, by reason of the absence of specificity of its action with respect to various spasm-inducing substances studied. This musculotropic action is exercised on the smooth fiber of the intestine as well as on the Oddi sphincter.

DISCUSSION

The results set forth above show that the acute toxicity of farnesol for the animals studied is low, and that it depends on the manner of administration and the excipient.

Observation of the behavior of the animals, at the time of study of this toxicity, leads one to believe that this substance is sedative for the central nervous system. This indication is confirmed by the study of fish, cited elsewhere, and by the hypothermia test in the Rat exposed to the cold.

The place of farnesol among the neurosedatives is clarified by examination of the results obtained with some standard tests. With respect to the three among them in which the animal is placed in a physical situation that is "acceptable" but that stimulates its psyche (fighting fish, actography, curiosity test), this terpene alcohol is shown to be clearly sedative, even at doses far from the LD_{50}. The same is not true for the tests in which the animal is placed in a physically "uncomfortable" situation (catalepsy, pull-up test, chimney test). These three tests bring into play a psychic component (surprise, discomfort, fright, flight instinct...) and a neuromuscular component (muscular tonus, agility, motor coordination...), but with respect to the first two in particular, farnesol proves to be inactive or scarcely active, and then only in the vicinity of toxic doses.

In vertebrates, farnesol therefore seems to be a sedative fairly specific for certain forms of excessive psychic activity (aggressiveness, curiosity), but which does not really depress the psychomotor defense activity except at high doses.

The pharmacological synergy or antagonism tests reported here confirm and clarify this opinion. Stimulation of the psyche by caffeine, demonstrated by an increase in motility or curiosity, is inhibited by farnesol which is inactive with respect to the spasms induced by pentetrazol. Finally, the potentiation of barbituric sleep supports the preceding notions. With the two methods, farnesol, nonhypnotic by itself, clearly intensifies the hypnogenic action of the barbiturates.

As regards the smooth muscular fiber, the tests presented here show that at low dose, farnesol is spasmolytic, an acetylcholine, barium chloride, histamine and serotonin antagonist. This absence of specificity with respect to the various spasm-inducing substances studied, suggests that this spasmolytic action is predominantly musculotropic. It is exercised on the intestine as well as on the Oddi sphincter.

In conclusion, it may be considered that in Vertebrates, farnesol is a psycho-sedative and a spasmolytic. Admittedly, the active doses are quite high (approximately 100 mg/kg), in excess of those observed with the medications endowed with these

properties. But what seems significant to us is that farnesol, a natural terpene alcohol, already known as a component of essential plant oils, as a metabolic intermediary in the synthesis of cholesterol, and similar to the neotenin of insects **[10]**, additionally offers psycho-sedative and spasmolytic properties in Vertebrates.

SUMMARY

In Vertebrates, farnesol, obtained by synthesis, is a psycho-sedative acting in particular against excessive psychic stimulation, that affects the psychmotor defense reactions only at high dose.

It is an antagonist of the stimulant action of caffeine, a potentiator of the hypnotic action of barbiturates, without being hypnotic itself.

Finally, it is a predominantly musculotropic spasmolytic, acting on the smooth fibers of the intestine as well as on the Oddi sphincter.

Work of the University of Paris-South, Faculty of Pharmaceutical and Biological Sciences, with the collaboration of the Research Department of MERAM *Laboratories (Melun).*

BIBLIOGRAPHY

[in languages of origin]

[items 2 and 3] à paraître = forthcoming

SUMMARY

Farnesol, obtained by synthesis, is a psycho-sedative acting in particular against excessive psychic stimulation.

It is an antagonist of the stimulant action of caffeine, it increases the hypnotic action of barbiturates, without being hypnotic itself.

It is a predominantly musculotropic spasmolytic.